## REMARKS

### I. Support for the Amendments

Claims 1-3 and 8-10 have been amended, and new claims 18 and 19 have been added. Previously withdrawn, non-elected claims 4, 5, 13, and 14 have been canceled without prejudice to their pursuit in an appropriate divisional or continuation application. Claims 1-3, 6-12, and 15-19 are currently in the application.

Support for claims 1-3 and 8-10, as amended, and for new claims 18 and 19 can be found throughout the original specification as filed. Additional support for new claim 18 can be found in the language of claims 1 and 9 as originally filed. Additional support for new claim 19 can be found in the language of claims 10 and 17 as originally filed.

More particularly, additional support for amended claims 1-3 and 8-10 and for new claim 18 can be found, e.g., on p. 4, ll. 5-17; from p. 4, l. 28, to p. 5, l. 3; from p. 6, l. 29, to p. 7, l. 9; on p. 10, ll. 1-19; and in the Examples. Additional support for amended claim 10 and for new claim 19 can be found, e.g., on p. 5, ll. 18-23; and on p. 10, ll. 21-30. Additional support for new claims 18 and 19 can be found, e.g., from page 11, l. 1, to page 12, l. 2.

### II. Status of the Claims

Claims 1-17 were originally filed with the application and were subject to a restriction requirement. Claims 1, 6-10, and 15-17 were stated by the Examiner to be generic. In the Response to the Election/Restriction Requirement, Applicants elected Group II species vascular endothelial growth factor (VEGF). The Examiner requested Applicants

to identify claims readable on this species. Applicants noted that "[t]hese generic claims and claims dependent thereon are readable on Group II" species.

Previously withdrawn, non-elected claims 4, 5, 13, and 14 have been canceled without prejudice to the filing of one or more divisional applications. New claims 18 and 19 have been added.

Claims 1-3, 6-12, and 15-19 are currently under examination, with claims 1 and 10 being the independent claims. Claims 2, 3, 6-9, and 18 are dependent on claim 1 or on claims dependent on claim 1, while claims 11, 12, 15-17 and 19 are dependent on claim 10 or on claims dependent on claim 10.

Applicants note that the present Office Action is non-final.

## III. The Supplemental Information Disclosure Statement Has Been Acknowledged

The Examiner has acknowledged all the references cited in the Supplemental Information Disclosure Statement. Applicants thank the Examiner for acknowledging the references.

## IV. The Objection to the Drawings is Traversed

In the Office Action mailed July 28, 2003, the U.S. Patent & Trademark Office had objected to the drawings, specifically to Figure 1. The Patent Office had stated:

> Figure 1 comprises 1A-1C. The drawings are objected to because the drawing descrption only describes Figure 1 but fails to acknowledge each individual

> drawing. A proposed drawing correction or corrected drawings are required in reply to the Office Action to avoid abandonment of the application. The objection to the drawings will not be held in abeyance. (P. 2.)

Figures 1A-1C were originally on a single page, entitled "Figure 1." In reply, Applicants filed corrected Figures 1A-1C with the addition of a Figure 1 legend as shown in the Replacement Figures, which were submitted concomitantly with the Amendment filed December 23, 2003. In the event that the Replacement Figures did not meet with the approval of the Examiner or of the Draftsperson, Applicants had indicated that they would be willing to amend the description of the drawings in the specification to replace [[Figure 1]] with --Figures 1A-1C--.

In the present Office Action, the Patent Office alleges:

> In response to the drawing objection, Applicants proposed to add a Figure 1 legend. However, this legend is not present in the attached Replacement Figures. The drawings filed on 9/21/2001 contain two sets of drawings. It is unclear which one is for official record. The objection will be withdrawn when Applicants clarify these matters and amend the description of the drawings in the specification to replace "Figure 1" with "Figures 1A-1C." [P. 2.]

In a telephone conference with the Examiner on July 15, 2004, it was noted that the figures originally filed with the application consisted of Figures 1-7 on 7 sheets (informal drawings), which were filed with the application on September 21, 2001. On May 21, 2002, formal Figures 1-7 on 9 sheets were filed with the Response to the Notice to File Missing Parts. Figure 1 (A-C) was now on three (3) sheets, rather than on one (1) sheet. On December 23, 2003, three (3) sheets of Figure 1 (A-C with legend in upper left-hand corner of Figure 1/1A) were filed with the previous Amendment in response to the previous Office Action.

The Examiner noted that the informal figures (7 sheets) and the set of formal figures (9 sheets) had been put together in the Patent Office file wrapper for a total of sixteen (16)

sheets, and she confirmed the presence of the legend on the replacement Figure 1 filed on December 23, 2003. The Examiner stated that there was no need to submit anything further with respect to figures at this time, but requested a notation in the present Amendment.

Applicants respectfully request confirmation of the acceptance of replacement Figure 1 (A-C) filed on December 23, 2003, and formal Figures 2-7 filed on May 21, 2002.

## V. Rejection Under 35 U.S.C. §112, Second Paragraph, is Accommodated

The Examiner has rejected claims 1-3, 6-12, and 15-17 under 35 U.S.C. §112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention."

More particularly, the Patent Office alleges:

> Claims 1-3, 6-12, 15-17 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: how to determine the ability of a gene encoding for an endothelial cell mitogen to produce a biologically active endothelial cell mitogen protein or the ability of a first plasmid DNA construct containing a gene encoding for an endothelial cell mitogen to produce a bioactive endothelial cell mitogen protein as compared to the ability of a second plasmid DNA construct containing a gene encoding for an endothelial cell mitogen to produce a bioactive endothelial cell mitogen protein.
>
> In response to this rejection, applicants argue that the amended claims overcome this rejection. This argument has been fully considered but deemed unpersuasive. As indicated in the previous office action, the method claims are required to refer back to the preamble so that there should have no gap between the steps and what the method is going to achieve. Since the claims are drawn to a method for testing a plasmid containing a gene encoding for an endothelial cell mitogen for the ability to produce a biologically active endothelial cell mitogen protein or a method for evaluating the ability of a first plasmid DNA construct containing a gene encoding for an endothelial cell mitogen to produce a bioactive endothelial cell mitogen protein as compared to the ability of a second plasmid DNA construct containing a gene encoding for an endothelial cell

> mitogen to produce a bioactive endothelial cell mitogen protein, such methods should comprise a step such as "wherein the level of cell survival indicate said plasmid produces an active endothelial cell mitogen" to make the method complete. The present claims do not have a step that refers back to the preamble of the method, thus the method is not complete. Therefore, the rejection is maintained. [P. 3.]

Applicants hereby amend claims 1 and 10 in accordance with the Examiner's remarks and have also amended claims 2, 3, 8, and 9 to be consistent with the amendments to claims 1 and 10. (Claims 6, 7, 11, 12, and 15-17 are dependent on either claim 1 or claim 10 or on claims dependent thereon.)

Applicants respectfully submit that the amendments to claims 1-3 and 8-10 overcome the Examiner's rejection of claims 1-3, 6-12, and 15-17 under 35 U.S.C. §112, second paragraph. Moreover, Applicants respectfully submit that the present claims 1-3, 6-12, and 15-17 fulfill the requirements of 35 U.S.C.§112, second paragraph, and request the Examiner's reconsideration of these claims accordingly.

**VI. Rejection of Claims 1-3, 6-12, and 15-17 under 35 U.S.C. §103(a) over Sugihara, in view of Buttke and Breier, is Traversed**

The Examiner has rejected claims 1-3, 7-12, and 15-17 under 35 U.S.C. §103(a) "as being unpatentable over Sugihara et al., in view of Buttke et al. and Breier et al." We disagree.

The Patent Office alleges:

> The teaching of Sugihara et al. was discussed in the Office Action mailed on 7/28/03. However, Sugihara et al. do not teach using Cos-1 cell line as host cells expressing the endothelial mitogen protein and the conditioned media are collected from transiently transfected host cell line.

The teaching of Buttke et al. was discussed in the Office Action mailed on 7/28/03. Buttke et al. further teach that MTS assay measures both mitogen induced cell proliferation and cell viability. Buttke et al. demonstrate that cell proliferation measured by thymidine incorporation correlated with MTS assay during the initial period, whereas MTS assay is more indicative of the number of cell viable when DNA synthesis declined (see page 238, 1st col.). Buttke et al. thus conclude that it is of particular interest to compare MTS-formazan production with thymidine uptake in cell culture (see page 238, 1st col., 1st paragraph, lines 14-15). Moreover, Buttke et al. show that MTS and labeled thymidine can be simultaneously added to cell cultures with each having little effect on the other. Since growth factors may either enhance cell viability or induce proliferation, Buttke et al. indicate that the ability to measure both MTS reduction and thymidine uptake in a single culture will be useful in studies pertaining to the isolation or characterization of novel growth factors (see page 239, 2nd col., last paragraph). [P. 5.]

With respect to Breier, the Patent Office alleges:

Breier et al. teach that conditioned media following transient transfection of expression vector comprising VEGF cDNA to Cos-1 cells are collected and assayed for mitogenic activity on bovine aortic endothelial cells (see page 524, 2nd col., 2nd paragraph, and page 522, Material and Methods, 1st paragraph). [P. 5.]

Finally, the Patent Office concludes:

It would have been obvious to one of ordinary skill of art to develop a method of testing the biological activity of a endothelial cell mitogen protein such as VEGF by measuring the survival rate of endothelial cells incubating with conditioned media collected from host cells transfected with vector encoding a endothelial cell mitogen based on the teaching of Sugihara et al (claims 1-3, 7-12, 16 and 17). Methods of measuring cell survival are well known in the art. Such methods include MTS/formazan assay taught by Buttke et al. One of ordinary skilled in the art would have been motivated to use MTS/formazan assay to measure cell survival because of the teaching of Burttke et al., who teach that MTS measures a cell survival, rather than cell proliferation as compared to thymidine incorporation. In addition, MTS/formazan and labeled thymidine can be added to cell culture simultaneously, then both parameters can be measured at the same time to determine whether a given factor affect proliferation or cell survival. Given the advantage of this assay, it would have been obvious to one of ordinary skill of art to use it in the mitogen assay taught

by Sugihara et al. to measure cell survival. Although the assay taught by Sugihara et al uses conditioned media from a stabled transfected cell line, one of ordinary skill in the art would recognize that conditioned media from host cells transiently transfected with a plasmid encoding the mitogen protein can also accomplish this task because method of transient transfection and stable transfection can be used interchangeably for the purpose of a mitogenic assay. **For example, Breier et al. teach a method for assaying mitogenic activity of VEGF on bovine aortic endothelial cells, in which it uses conditioned media from Cos-1 cells transiently transfected with expression vector expressing VEGF.** The level of skill in the art is high. Absent evidence from the contrary, one of ordinary skill of art would have reasonable expectation of success to develop a method of testing the biological activity of a endothelial cell mitogen protein by transiently transfecting the expression vector to a host cell line, incubating endothelial cell with the conditioned media from the transiently transfected host cell line, and determine cell survival by MTS assay. Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill of art at the time the invention was made.

It would also have been obvious to one of ordinary skill of art to use the method taught by Sugihara et al. and using Cos-1 cell as host cell for expressing endothelial cell mitogen protein because both NIH3T3 cells and Cos-1 cells are widely used in transfection experiment and expressing a protein of interest (claims 6 and 15). The ordinary artisan would have been motivated to use either Cos-1 or NIH3T3 as host cells because **both Sugihara et al. and Breier et al. teach that conditioned media from either cell line transfected with endothelial mitogen protein promotes endothelial cell growth.** The level of skill in the art is high. Absent evidence to the contrary, one of ordinary skill in the art would have reasonable expectation of success to use Cos-1 cells to express a biological active endothelial mitogen protein. Therefore, the invention would have been *prima facie* obvious to one of ordinary skill of art at the time the invention was made. [Pp. 5-7; italics in original; bold emphasis added.]

Applicants respectfully disagree with these comments and traverse the obviousness rejection.

With respect to Sugihara and as noted in the Amendment filed December 23, 2003, Applicants and the Patent Office are in agreement that the transfection assay method described therein would appear to involve stable, rather than transient, transfection and that Sugihara does not teach using Cos-1 cell line as host cells expressing the endothelial mitogen protein.

Applicants also noted that **Sugihara describes a cell mitogenic assay using incorporation of $^{3}$H-thymidine during the cell cycle as a means of measuring cell proliferation**. In contrast, **the present invention provides a method for testing the survival of cells**, that is, their ability to overcome cell death, **as measured by a cell viability assay**. These **traits are quite different**, because viable cells are not necessarily undergoing mitosis. **Sugihara does not teach measuring cell survival by tetrazolium (MTS)/formazan assay** or by any other assay, because **Sugihara does not teach measuring of cell survival at all**.

With respect to Buttke, as noted by Applicants in the Amendment filed December 23, 2003, this reference teaches an MTS/formazan assay (see page 12 of the specification). Applicants submit, however, that **Buttke repeatedly distinguishes between the use of the $^{3}$H-thymidine assay to measure cell proliferation and the use of the MTS/formazan assay to measure cell viability**. Moreover, Buttke repeatedly emphasizes **the use of both assays to distinguish between cell proliferation and cell viability**, such as those described on page 238 with the FDC-P1 cell line.

Because **the cell proliferation assay of Sugihara and the cell viability assay of Buttke are measuring two different parameters, Buttke cannot supply the deficiencies of Sugihara**. There is **no motivation** for one of skill in the art **to use the stable transfection technique and cell proliferation assay** of Sugihara **to perform the transient transfection and cell viability assay** of the present invention. The cell proliferation assay described in Sugihara does not necessarily provide a measurement of cell viability as provided according to the present invention or as provided in Buttke. Moreover, there **is no suggestion in Sugihara** that measurement of cell viability, as opposed to cell proliferation, would be desirable.

Likewise, Buttke not only fails to remedy the deficiencies of Sugihara, but there is **no suggestion in Buttke that measurement of cell proliferation would necessarily be interchangeable with measurement of cell viability**. While Buttke provides instances in which cell proliferation and viability coincide, Buttke also provides **an example**, described above, in which **cell proliferation ceases while cell viability continues**.

In essence, therefore, **Buttke teaches away from Sugihara** by emphasizing a marked preference for **the need for both tests** as a means of **measuring and comparing two different cell parameters**, including an example in which **the results of the two assays differed precisely because they were measuring two different parameters**. As a result, **any technical advantages** of the MTS/formazan assay over the $^{3}$H-thymidine assay are **essentially irrelevant**, because the assays are **measuring two different cell parameters**.

Finally, Breier cannot supply the deficiencies of Buttke and Sugihara. The Patent Office alleges that "Breier et al. teach that conditioned media following transient transfection of expression vector comprising VEGF cDNA to Cos-1 cells are collected and assayed for mitogenic activity on bovine aortic endothelial cells (see page 524, 2nd col., 2nd paragraph, and page 522, Material and Methods, 1st paragraph)," but the passages cited by the Patent Office do not discuss cell survival.

In the Materials and Methods section, Breier states:

> *Cell culture, transfection and bioassays*
> COS-1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS). Cells were transfected using the modified calcium phosphate method (Choen and Okayama, 1987). Conditioned media were collected 48-72 hours after transfection. Bovine aortic endothelial (BAE) cells were prepared as described by Schwartz (1978) and grown in DMEM supplemented with 5% FCS. Assays for mitogenic activity were performed as described (Risau et al., 1988). In short, BAE cells (passages 9-12) were seeded in 24-well dishes at a density of 10 000 cells per well the day before addition of the COS cell-conditioned media. After three days, **the cells were**

**dissociated with trypsin and counted in a Coulter counter.** [P. 522, 1st par.; italics in original; bold emphasis added.]

In the subsection entitled, "Secretion and mitogenic activity of recombinant murine VEGF" (p. 524, 2nd col., 2nd par.), Breier states, in pertinent part:

> *Secretion and mitogenic activity of recombinant murine VEGF*
> The VEGF cDNAs were cloned into a mammalian expression vector which contained the transcriptional control elements of the human cytomegalovirus. The resulting plasmids were then transfected into COS cells. Culture supernatants and extracts prepared from the transfected cells were assayed by immunoblot analysis for the presence of VEGF protein....Homodimers...were detected in the conditioned media, corresponding to VEGF-1 or to VEGF-2 polypeptide chains, respectively (Fig. 4). In contrast, the VEGF-3 chains were retained in the cells....The cell culture supernatants were then assayed for mitogenic activity on bovine aortic endothelial (BAE) cells. **The conditioned media which contained homodimers of VEGF-1 or VEGF-2 stimulated the <u>proliferation</u> of BAE cells** to a similar extent (Fig. 5). Media from cells transfected with VEGF-3 cDNA had **no significant mitogenic effect**. Whether VEGF-3 protein can be as active as VEGF-1 or VEGF-2 in **stimulating the <u>proliferation</u> of endothelial cells** remains to be determined. [P. 524, 2nd col., 2nd par.; italics in original; bold and underlined emphasis added.]

In essence, 1) Breier equates "mitogenic effect" with "stimulating the proliferation of endothelial cells" and 2) Breier's assay, as described within the four corners of the Breier reference, merely refers to counting cells "in a Coulter counter" to measure "proliferation of endothelial cells."

This view is borne out by Fig. 5, which is simply a bar graph of the number of cells per well, as described above and in the figure legend, which reads:

> **Fig. 5.** <u>Mitogenic activity</u> of recombinant VEGF. Bovine aortic endothelial cells were cultured in the presence or absence of cell culture supernatants from transfected COS cells (see Fig. 4). DMEM supplemented with 10% FCS and the conditioned media from untransfected COS cells or from COS cells that were transfected with the vector alone were used as a negative control. After three days, <u>the cells were dissociated with trypsin and counted in a cytometer</u>. Values are means ± s.e.m. of double determinations. [P. 525; bold in original; underline emphasis added.]

Similar to Sugihara, Breier **describes a cell mitogenic assay using a Coulter counter to count cells as a means of measuring cell proliferation**. In contrast, **the present invention provides a method for testing the survival of cells**, that is, their ability to overcome cell death, **as measured by a cell viability assay**. These **traits are quite different**, because viable cells are not necessarily undergoing mitosis. Breier never distinguishes between cells at different stages of mitosis. **A mere cell count cannot distinguish between the two parameters of cell viability and cell proliferation. Breier does not teach measuring cell survival by simply counting the number of cells** or by any other assay, because **Breier does not teach measuring of cell survival at all.**

Buttke uses the **$^{3}$H-thymidine assay, rather than the Coulter counter, to measure cell proliferation**. Buttke not only fails to remedy the deficiencies of Breier, but there is **no suggestion in Buttke that measurement of cell proliferation would necessarily be interchangeable with measurement of cell viability**. While Buttke provides instances in which cell proliferation and viability coincide, Buttke also provides **an example**, described above, in which **cell proliferation ceases while cell viability continues**.

In essence, similar to Sugihara, therefore, **Buttke teaches away from Breier** by emphasizing a marked preference for **the need for measuring and comparing two different cell parameters**.

Moreover, there is no suggestion in Breier to combine the teachings of Breier with those of Sugihara and/or Buttke. Like Sugihara, Breier measures cell proliferation – not cell viability. Breier does not distinguish between the two parameters. As a result, the use of COS-1 cells in the Breier assay is irrelevant, because the parameters being measured are different.

Applicants respectfully submit that the present claims 1-3, 6-12, and 15-17 fulfill the requirements of 35 U.S.C.§103(a) and request the Examiner's reconsideration of these claims accordingly.

## VII. Conclusion

In view of the foregoing amendments and remarks, the present application is respectfully considered in condition for allowance. An early reconsideration and notice of allowance are earnestly solicited.

It is believed that all outstanding rejections have been addressed by this submission and that all the claims are in condition for allowance. If discussion of any amendment or remark made herein would advance this important case to allowance, the Examiner is invited to call the undersigned as soon as convenient.

Applicants hereby request a three-month extension of time for the Amendment and accompanying materials and hereby submit the requisite fee accordingly. If a petition for an additional extension of time is required, then the Examiner is requested to treat this as a conditional petition for an additional extension of time. Although it is not believed that any additional fee (in addition to the fee concurrently submitted) is required to consider this submission, the Commissioner is hereby authorized to charge our deposit account no. 04-1105 should any fee be deemed necessary.

Respectfully submitted,

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